REMARKS

Claims 1, 4, 42, 43, and 45-56 are pending. Claims 1, 4, 42, 43, and 45-56 are rejected under 35 U.S.C. § 112, first paragraph, and claims 1, 43, and 45-56 are rejected under 35 U.S.C. § 102. Applicants address each of these rejections as follows.

Claim Amendment

Claim 51 has been amended to require an antibody that specifically binds the tumor-specific N-linked glycostructure of the section to induce apoptosis of a cell expressing a glycosylated human CD55 protein containing the tumor-specific N-linked glycostructure. This amendment finds support, for example, at page 4, lines 15-21, of the English language specification. No new matter has been added by the present amendment.

Rejection under 35 U.S.C. § 112, first paragraph

Claims 1, 4, 42, 43, and 45-56 are rejected under 35 U.S.C. § 112, first paragraph, as failing to comply with the written description requirement. The Office asserts (page 4):

> The crux of the rejection is Applicants' inability to define the tumor-specific N-linked glycostructure and how one of ordinary skill in the art could identify the said structure [sic] ... Not knowing or not being able to clearly define the tumor specific N-linked glycostructure, which is germane to the claimed invention, implies that Applicants have not adequately described the species and consequently are not in possession of the genus. In view of Applicants not being able to define, nor characterize the glycostructure one of ordinary skill in the art is not clear on the variability

that possibly exists within the genus of glycoproteins.

* * *

There is a plethora of species that could be encompassed by the broad claims.

Applicants respectfully traverse this ground for rejection.

The claimed invention does not read on a genus of glycoproteins with undefined glycostructures, but rather to a particular isolated glycoprotein with a particular glycostructure and sections of the glycoprotein having the particular glycostructure. Both the glycoprotein and the glycostructure, as described in Applicants' specification, meet the written description requirement of 35 U.S.C. § 112, first paragraph.

The glycostructure

Claim 1 is directed to an isolated glycoprotein containing the human amino acid primary structure of CD55 and a tumor-specific N-linked glycostructure. The claim further requires the glycoprotein to have an apparent molecular weight of about 82 kD and to be a glycoprotein present on adenocarcinoma cell line 23132, but not on a normal cell. Similarly, the glycoprotein of claim 50 is required to contain a section of a glycosylated human CD55 protein expressed by adenocarcinoma cell line 23132, but not by a normal cell. The glycosylated human CD55 protein has an apparent molecular weight of about 82 kD, and the section of the glycosylated human CD55 protein includes a tumor-specific N-linked glycostructure. The specification describes the 23132 cell line

as being deposited in a public depository[1]. In particular, at page 5, lines 12-18, of the English language text, the specification states:

> [S]uch a glycoprotein that can be obtained from, for example, human adenocarcinoma cell line 23132 (DSM ACC 201) ... has an apparent molecular weight of about 82 kD.

Applicants note that, in *Enzo Biochem*, the Federal Circuit has held that one may comply with the written description requirement by publicly depositing the biological material. The Court stated:

> [R]eference in the specification to a deposit in a public depository, which makes its contents accessible to the public when it is not otherwise available in written form, constitutes an adequate description of the deposited material sufficient to comply with the written description requirement of § 112, ¶ 1.

Enzo Biochem, Inc., v. Gen-Probe Inc., 296 F.3d 1316, 1325 63 U.S.P.Q.2d 1609, 1613 (Fed. Cir. 2002).

In *Enzo Biochem*, the deposits were recombinant bacterial cells expressing the DNA molecules of interest. Given that Applicants' specification describes a publicly deposited cell line expressing a glycoprotein having the glycostructure encompassed by the present claims, Applicants submit that the description of the glycostructure in Applicants' specification meets the standard set forth by the Federal Circuit in *Enzo Biochem*. On this basis alone, the glycostructure recited in the present claims finds sufficient written description in the specification as filed to meet the requirements of 35 U.S.C. § 112, first paragraph.

[1] Applicants note that the DSMZ is included in the list of acceptable depositories provided in M.P.E.P. § 2405.

In addition, antibodies that recognize the amino acid primary structure of CD55 (DAF) were also available in the art at the time the present application was filed (see, e.g., Hara et al., Immunol. Lett. 37:145-152, 1993; copy enclosed with Applicants' August 30, 2004 reply). In fact, Karnauchow et al. (Journal of Virology 70:5143-5152, 1996; hereafter "Karnauchow") cited by the Office in the present Office Action describes an antibody that binds wild-type CD55 (DAF). These publicly available antibodies allow one skilled in the art to identify and isolate the CD55 glycoprotein expressed by the 23132 cell line. For all the above reasons, there can be no question that, at the time of filing, Applicants were in possession of the glycostructure recited in the present claims.

The amino acid primary structure

Claim 1 requires the glycoprotein to contain the human amino acid primary structure of CD55. This claim limitation refers to the amino acid sequence of a known protein. The nucleic acid and amino acid sequences of wild-type CD55 (DAF) were publicly known at the time the present application was filed (see, e.g., Caras et al., U.S. Patent No. 5,763,224, issued June 9, 1998, and entitled "Decay Accelerating Factor (DAF) and Nucleic Acids Encoding It; copy enclosed with Applicants' August 30, 2004 reply).

With regard to the description of the amino acid primary structure of CD55, Applicants direct the Office's attention to a recent decision by the United States Court of Appeals for the Federal Circuit, *Capon v. Eshhar,* 418 F.3d 1349, 76 U.S.P.Q. (BNA)

1078 (Fed. Cir. 2005); a copy of which is provided herewith as Exhibit A. The *Capon* decision is based on an interference proceeding involving claims directed to chimeric genes for the production of membrane-bound proteins. The Board of Patent Appeals and Interferences ("the Board") rejected the claims of both parties under 35 U.S.C. § 112, first paragraph, noting that the claims lacked an adequate written description in the specification. In particular, the Board concluded that the written description requirement necessitated a listing of the specific nucleotide sequences of the claimed DNA. On appeal, the Federal Circuit reversed, with the court stating:

> The chimeric genes here at issue are prepared from known DNA sequences of known function. The Board's requirement that these sequences must be analyzed and reported in the specification does not add descriptive substance. The Board erred in holding that the specifications do not meet the written description requirement because they do not reiterate the structure or formula or chemical name for the nucleotide sequences of the claimed chimeric genes. (Emphasis added.)

Capon, 418 F.3d at 1358.

The written description requirement must be applied in the context of the particular invention and the state of the knowledge in the art.

The disputed claims in *Capon* were directed to chimeric genes encoding specific domain components defined in terms of their function and origin rather than their specific structure. The Board found the functional limitations to be insufficient, noting that controlling precedent required the inclusion in the specification of the complete nucleotide sequence of at least one chimeric gene as well as reference to "structure, formula, chemical name, or physical properties" of the many protein domains, and/or

DNA sequences which encode many protein domains referenced in the claims. As noted above, the Federal Circuit stated that "the Board erred in ruling that § 112 imposes a *per se* rule requiring recitation in the specification of the nucleotide sequence of claimed DNA, when that sequence is already known in the field." *Capon,* 418 F.3d at 1360.

The presently claimed invention is based on the finding that a glycoprotein having the human amino acid primary structure of CD55 and a particular glycostructure is expressed by tumor cells, but not by normal cells. Following the principles set forth in the *Capon* decision, the amino acid primary structure of CD55 need not be reiterated, described, or reproduced in the instant specification to comply with the written description requirement of 35 U.S.C. § 112, first paragraph.

With respect to claim 50, Applicants note that, as shown in Figure 7 of Coyne et al. (J. Immunology 149:2906-2913, 1992; "Coyne;" copy enclosed with Applicants' August 30, 2004 reply), CD55/DAF only contains one N-linked glycosylation site. Given that the sequence of wild-type CD55 and the location of its single N-linked glycosylation site were publicly known at the time the application was filed, one skilled in the art would recognize which section of CD55 would contain a tumor-specific N-linked glycostructure. Thus, like the amino acid sequence of the human CD55 primary structure, Applicants submit that a description of the only N-linked glycosylation site need not be included in the specification to adequately describe the sections recited in the present claims.

Moreover, claim 50 clearly limits which sections of the CD55 can be included in the claimed glycoprotein. The sections are required to be sections of the 82 kD CD55 protein expressed by publicly deposited cell line 23132 that contain the tumor-specific N-linked glycostructure. As CD55 only contains one N-linked glycosylation site, all sections encompassed by claim 50 must include this glycosylation site.

In sum, in view of the above Federal Circuit decisions, Applicants submit that reference in the specification as filed to a deposit of a cell line expressing a glycoprotein having the glycostructure recited in the present claims provides adequate written description for the glycostructure. Also, given that the sequence of the amino acid primary structure of CD55 and the location, in this sequence, of the single N-linked glycosylation site were known and publicly available at the time of filing, Applicants need not provide these sequences in the specification to adequately describe the CD55 sequences encompassed by the present claims. The 35 U.S.C. § 112, first paragraph, rejection should be withdrawn.

Rejection under 35 U.S.C. § 102

Claims 1, 43, and 45-56 are rejected under 35 U.S.C. § 102(b) as being anticipated by Karnauchow et al. (Journal of Virology 70:5143-5152, 1996; "Karnauchow"). In particular, the Office asserts (page 5):

> The glycoprotein of Karnauchow is found on a tumor cell, Hela cell, which is not a normal cell and the glycoprotein is within the range of "about 82 kD."

Applicants disagree.

Applicants submit that the glycoprotein described by Karnauchow does not have a molecular weight of about 82 kD as required by the claims. Karnauchow states (page 5143, right column):

> Human DAF is a 70- to 75-kDa membrane glycoprotein involved in protecting cells against lysis by homologous complement.

And (page 5146, right column):

> Mab EVR1 reacted specifically with a HeLa cell protein of approximately 70 to 75 kDa that appeared to migrate as a doublet in polyacrylamide gels.

70 or 75 kD are not within the range of about 82 kD. The specification teaches that Applicants were able to readily distinguish between proteins of approximately 70 kD and approximately 82 kD (see, e.g., page 28, lines 12-22, of the English language specification). Here the specification teaches:

> By altering stringency (1M of NaCl) and with use of membrane preparations, it was possible to detect other proteins with approximately 70 kD and approximately 82 kD (Figure 1a, trace 1).

Figure 1A shows a Western blot with several distinct protein bands. The 70 kD band is clearly distinguished from the 82 kD band. Moreover, the specification describes 70 kD protein as being the "human Lupus p70 auto-antigen protein (gene bank access no. J04611)." Consistent with Applicants' findings, the art also describes the human Lupus p70 auto-antigen as having a molecular weight of approximately 70 kD, as evidenced by Reeves and Sthoeger (J. Biol. Chem. 264:5047-5052, 1989; "Reeves;" enclosed as Exhibit B). In fact, Reeves readily distinguishes the 70 kD human Lupus auto-antigen

from an 80 kD protein (see, e.g., Figure 3). Clearly a 70 kD or 75 kD protein does not have a molecular weight of approximately 82 kD. Applicants submit that Karnauchow does not describe a glycoprotein of approximately 82 kD and, therefore, does not describe a glycoprotein that meets all of the limitations of the present claims. The anticipation rejection of claims 1 and 50 over Karnauchow should be withdrawn.

The Office also asserts:

> The disclosed antibody is specific for the glycostructure and consequently is capable of presenting the results listed in claims 43, 45-49 and 51-56.

Applicants disagree.

Claim 43 requires that the glycoprotein, if present on a cell and bound by an antibody specific for said glycostructure, results in apoptosis of the cell. Claim 51 requires that an antibody that specifically binds the tumor-specific N-linked glycostructure of the section, upon binding, induces apoptosis of a cell expressing the glycosylated human CD55 protein. The antibody described by Karnauchow does not inducing apoptosis of HeLa cells or of cells recombinantly expressing DAF (CD55). In fact, Karnauchow teaches that EVR1 can bind to HeLa cells and protect them from Enterovirus 70 (EV70) infection. In particular, Karnauchow states (page 5146, left column):

> Undiluted EVR1 hybridoma supernatant completely protected HeLa cell monolayers against EV70 infection, with monolayers remaining free of CPE [virus-induced cytopathic effects] for 96 h, at which time monitoring was terminated. Neither HeLa nor LLC-MK_2 cells were protected against poliovirus or coxsackievirus B3. In all cases, the monolayers were completely destroyed within 18 h following infection.

Clearly, HeLa cells bound by EVR1 did not undergo apoptosis, but survived as a monolayer for more than 96 hours. Accordingly, given that the effect observed by Karnauchow is opposite to that required by claims 43 and 51 (cell survival vs. apoptosis), Karnauchow does not teach every feature of the invention of claims 43 and 51, this reference cannot anticipate claims 43 and 51 and their dependent claims.

CONCLUSION

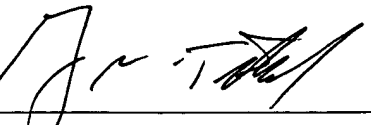
Applicants submit that the application is now in condition for allowance, and this action is hereby respectfully requested.

Enclosed is a Petition to extend the period for replying to the final Office Action for three (3) months, to and including December 5, 2005 (December 3, 2005 falls on a Saturday), and a check in payment of the required extension fee.

If there are any additional charges or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

Date: 5 December 2005



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1 of 1 DOCUMENT

DANIEL J. CAPON, ARTHUR WEISS, BRIAN A. IRVING, MARGO R. ROBERTS, and KRISZTINA ZSEBO, Appellants, v. ZELIG ESHHAR, DANIEL SCHINDLER, TOVA WAKS, and GIDEON GROSS, Cross-Appellants, v. JON DUDAS, Director of the Patent and Trademark Office, Intervenor.

03-1480, 03-1481

UNITED STATES COURT OF APPEALS FOR THE FEDERAL CIRCUIT

418 F.3d 1349; 2005 U.S. App. LEXIS 16865; 76 U.S.P.Q. (BNA) 1078

August 12, 2005, Decided

PRIOR HISTORY: [**1] Appealed from: United States Patent and Trademark Office Board of Patent Appeals and Interferences. (Interference No. 103,887)

LexisNexis(R) Headnotes

COUNSEL: Steven B. Kelber, Piper Rudnick, LLP, of Washington, DC, argued for appellants.

Roger L. Browdy, Browdy and Neimark, P.L.L.C., of Washington, DC, argued for cross-appellants.

Mary L. Kelly, Associate Solicitor, Office of the Solicitor, United States Patent and Trademark Office, of Arlington, Virginia, argued for intervenor. With her on the brief were John M. Whealan, Solicitor and Stephen Walsh, Associate Solicitor.

JUDGES: Before NEWMAN, MAYER, * and GAJARSA, Circuit Judges.

* Haldane Robert Mayer vacated the position of Chief Judge on December 24, 2004.

OPINIONBY: NEWMAN

OPINION: [*1350] NEWMAN, Circuit Judge.

Both of the parties to a patent interference proceeding have appealed the decision of the Board of Patent Appeals and Interferences of the United States Patent and Trademark Office, wherein the Board held that the specification of neither party met the written description requirement of the patent statute. Capon v. Eshhar, Interf. No. 103,887 (Bd. Pat. App. & Interf. Mar. 26, 2003). The Board dissolved the interference and cancelled all [**2] of the claims of both parties corresponding to the interference count. With this ruling, the Board terminated the proceeding and did not reach the question of priority of invention. We conclude that the Board erred in its application of the law of written description. The decision is vacated and the case is remanded to the Board for further proceedings.

BACKGROUND

Daniel J. Capon, Arthur Weiss, Brian A. Irving, Margo R. Roberts, and Krisztina Zsebo (collectively "Capon") and Zelig Eshhar, Daniel Schindler, Tova Waks, and [*1351] Gideon Gross (collectively "Eshhar") were the parties to an interference proceeding between Capon's *United States Patent No. 6,407,221* ("the *'221 patent*") entitled "Chimeric Chains for Receptor-Associated Signal Transduction Pathways" and Eshhar's patent application Serial No. 08/084,994 ("the '994 application") entitled "Chimeric Receptor Genes and Cells Transformed Therewith." Capon's *Patent No. 5,359,046* ("the '046 patent"), parent of the *'221 patent*, was also included in the interference but was held expired for non-payment of a maintenance fee. The PTO included the *'046* patent in its decision and in its argument of this appeal. n1

n1 Although Capon is designated as appellant and Eshhar as cross-appellant, both appealed the Board's decision. See *Fed. R. App. P. 28(h)*. The Director of the PTO intervened to support the Board, and has fully participated in this appeal.

[**3]

A patent interference is an administrative proceeding pursuant to *35 U.S.C. §§ 102(g)* and *135(a)*, conducted for the purpose of determining which of competing applicants is the first inventor of common subject matter. An interference is instituted after the separate patent applications have been examined and found to contain patentable subject matter. Capon's patents had been examined and had issued before this interference was instituted, and

Eshhar's application had been examined and allowed but a patent had not yet issued.

During an interference proceeding the Board is authorized to determine not only priority of invention but also to redetermine patentability. *35 U.S.C. § 6(b)*. The question of patentability of the claims of both parties was raised *sua sponte* by an administrative patent judge during the preliminary proceedings. Thereafter the Board conducted an *inter partes* proceeding limited to this question, receiving evidence and argument. The Board then invalidated all of the claims that had been designated as corresponding to the count of the interference, viz., all of the claims of the Capon *'221 patent*, claims 5-8 of [**4] the Capon *'046* patent, and claims 1-7, 9-20, and 23 of the Eshhar '994 application.

In accordance with the Administrative Procedure Act, the law as interpreted and applied by the agency receives plenary review on appeal, and the agency's factual findings are reviewed to determine whether they were arbitrary, capricious, or unsupported by substantial evidence in the administrative record. See *5 U.S.C. § 706(2)*; *Dickinson v. Zurko, 527 U.S. 150, 164-65, 144 L. Ed. 2d 143, 119 S. Ct. 1816 (1999)*; *In re Gartside, 203 F.3d 1305, 1315 (Fed. Cir. 2000)*.

The Invention

A chimeric gene is an artificial gene that combines segments of DNA in a way that does not occur in nature. The *'221 patent* and '994 application are directed to the production of chimeric genes designed to enhance the immune response by providing cells with specific cell-surface antibodies in a form that can penetrate diseased sites, such as solid tumors, that were not previously reachable. The parties explain that their invention is a way of endowing immune cells with antibody-type specificity, by combining known antigen-binding-domain producing DNA and known lymphocyte-receptor-protein [**5] producing DNA into a unitary gene that can express a unitary polypeptide chain. Eshhar summarized the problem to which the invention is directed:

> Antigen-specific effector lymphocytes, such as tumor-specific T cells, are very rare, individual-specific, limited in their recognition spectrum and difficult to obtain against most malignancies. Antibodies, on the other hand, are readily [*1352] obtainable, more easily derived, have wider spectrum and are not individual-specific. The major problem of applying specific antibodies for cancer immunotherapy lies in the inability of sufficient amounts of monoclonal antibodies (mAb) to reach large areas within solid tumors.

Technical Paper Explaining Eshhar's Invention, at 6.

The inventions of Capon and Eshhar are the chimeric DNA that encodes single-chain chimeric proteins for expression on the surface of cells of the immune system, plus expression vectors and cells transformed by the chimeric DNA. The experts for both parties explain that the invention combines selected DNA segments that are both endogenous and nonendogenous to a cell of the immune system, whereby the nonendogenous segment encodes the single-chain variable ("scFv") [**6] domain of an antibody, and the endogenous segment encodes cytoplasmic, transmembrane, and extracellular domains of a lymphocyte signaling protein. They explain that the scFv domain combines the heavy and light variable ("Fv") domains of a natural antibody, and thus has the same specificity as a natural antibody. Linking this single chain domain to a lymphocyte signaling protein creates a chimeric scFv-receptor ("scFvR") gene which, upon transfection into a cell of the immune system, combines the specificity of an antibody with the tissue penetration, cytokine production, and target-cell destruction capability of a lymphocyte.

The parties point to the therapeutic potential if tumors can be infiltrated with specifically designed immune cells of appropriate anti-tumor specificity.

The Eshhar Claims

The Board held unpatentable the following claims of Eshhar's '994 application; these were all of the '994 claims that had been designated as corresponding to the count of the interference. Eshhar's claim 1 was the designated count.

> 1. A chimeric gene comprising
>
> a first gene segment encoding a single-chain Fv domain (scFv) of a specific antibody and
>
> a second gene segment [**7] encoding partially or entirely the transmembrane and cytoplasmic, and optionally the extracellular, domains of an endogenous protein
>
> wherein said endogenous protein is expressed on the surface of cells of the immune system and triggers activation and/or proliferation of said cells,
>
> which chimeric gene, upon transfection to said cells of the immune system, expresses said scFv domain and said domains of said endogenous protein in one single chain on

the surface of the transfected cells such that the transfected cells are triggered to activate and/or proliferate and have MHC nonrestricted antibody-type specificity when said expressed scFV domain binds to its antigen.

2. A chimeric gene according to claim 1 wherein the second gene segment further comprises partially or entirely the extracellular domain of said endogenous protein.

3. A chimeric gene according to claim 1 wherein the first gene segment encodes the scFv domain of an antibody against tumor cells.

4. A chimeric gene according to claim 1 wherein the first gene segment encodes the scFv domain of an antibody against virus infected cells.

5. A chimeric gene according to claim 4 wherein the virus is HIV.

6. [**8] A chimeric gene according to claim 1 wherein the second gene segment encodes a lymphocyte receptor chain.

[*1353] 7. A chimeric gene according to claim 6 wherein the second gene segment encodes a chain of the T cell receptor.

9. A chimeric gene according to claim 7 wherein the second gene segment encodes the a, B, y, or o chain of the antigen-specific T cell receptor.

10. A chimeric gene according to claim 1 wherein the second gene segment encodes a polypeptide of the TCR/CD3 complex.

11. A chimeric gene according to claim 10 wherein the second gene segment encodes the zeta or eta isoform chain.

12. A chimeric gene according to claim 1 wherein the second gene segment encodes a subunit of the Fc receptor or IL-2 receptor.

13. A chimeric gene according to claim 12 wherein the second gene segment encodes a common subunit of IgE and IgG binding Fc receptors.

14. A chimeric gene according to claim 13 wherein said subunit is the gamma subunit.

15. A chimeric gene according to claim 13 wherein the second gene segment encodes the CD16a chain of the FcyRIII or FcyRII.

16. A chimeric gene according to claim 12 wherein the second gene segment encodes the a [**9] or B subunit of the IL-2 receptor.

17. An expression vector comprising a chimeric gene according to claim 1.

18. A cell of the immune system endowed with antibody specificity transformed with an expression vector according to claim 17.

19. A cell of the immune system endowed with antibody specificity comprising a chimeric gene according to claim 1.

20. A cell if the immune system according to claim 19 selected from the group consisting of a natural killer cell, a lymphokine activated killer cell, a cytotoxic T cell, a helper T cell and a subtype thereof.

23. A chimeric gene according to claim 1 wherein said endogenous protein is a lymphocyte receptor chain, a polypeptide of the TCR/CD3 complex, or a subunit of the Fc or IL-2 receptor.

The Board did not discuss the claims separately, and held that the specification failed to satisfy the written description requirement as to all of these claims.

The Capon Claims

Claims 1-10, all of the claims of the *'221 patent*, were held unpatentable on written description grounds. Claims 1-6 are directed to the chimeric DNA, claims 7, 8, and 10 to the corresponding cell comprising the DNA, and claim 9 to [**10] the chimeric protein:

1. A chimeric DNA encoding a membrane bound protein, said chimeric DNA comprising in reading frame:

DNA encoding a signal sequence which directs said membrane bound protein to the surface membrane;

DNA encoding a non-MHC restricted

extracellular binding domain which is obtained from a single chain antibody that binds specifically to at least one ligand, wherein said at least one ligand is a protein on the surface of a cell or a viral protein;

DNA encoding a transmembrane domain which is obtained from a protein selected from the group consisting of CD4, CD8, immunoglobulin, the CD3 zeta chain, the CD3 gamma chain, the CD3 delta chain and the CD3 epsilon chain; and

DNA encoding a cytoplasmic signal-transducing domain of a protein that activates an intracellular messenger system which is obtained from CD3 zeta,

[*1354] wherein said extracellular domain and said cytoplasmic domain are not naturally joined together, and said cytoplasmic domain is not naturally joined to an extracellular ligand-binding domain, and when said chimeric DNA is expressed as a membrane bound protein in a host cell under conditions suitable for expression, said membrane bound protein [**11] initiates signaling in said host cell when said extracellular domain binds said at least one ligand.

2. The DNA of claim 1, wherein said single-chain antibody recognizes an antigen selected from the group consisting of viral antigens and tumor cell associated antigens.

3. The DNA of claim 2 wherein said single-chain antibody is specific for the HIV env glycoprotein.

4. The DNA of claim 1, wherein said transmembrane domain is naturally joined to said cytoplasmic domain.

5. An expression cassette comprising a transcriptional initiation region, the DNA of claim 1 under the transcriptional control of said transcriptional initiation region, and a transcriptional termination region.

6. A retroviral RNA or DNA construct comprising the expression cassette of claim 5.

7. A cell comprising the DNA of claim 1.

8. The cell of claim 7, wherein said cell is a human cell.

9. A chimeric protein comprising in the N-terminal to C-terminal direction:

a non-MHC restricted extracellular binding domain which is obtained from a single chain antibody that binds specifically to at least one ligand, wherein said at least one ligand is a protein on the surface of a cell [**12] or a viral protein;

a transmembrane domain which is obtained from a protein selected from the group consisting CD4, CD8, immunoglobulin, the CD3 zeta chain, the CD3 gamma chain, the CD3 delta chain and the CD3 epsilon chain; and

a cytoplasmic signal-transducing domain of a protein that activates an intracellular messenger system which is obtained from CD3 zeta,

wherein said extracellular domain and said cytoplasmic domain are not naturally joined together, and said cytoplasmic domain is not naturally joined to an extracellular ligand-binding domain, and when said chimeric protein is expressed as a membrane bound protein in a host cell under conditions suitable for expression, said membrane bound protein initiates signaling in said host cell when said extracellular domain binds said at least one ligand.

10. A mammalian cell comprising as a surface membrane protein, the protein of claim 9.

In addition, claims 5, 6, 7, and 8 of Capon's '*046* patent were held unpatentable. These claims are directed to chimeric DNA sequences where the encoded extracellular domain is a single-chain antibody containing ligand binding activity.

The Board Decision

The Board presumed [**13] enablement by the specifications of the *'221 patent* and '994 application of the full scope of their claims, and based its decision solely on the ground of failure of written description. The Board held that neither party's specification provides the requisite description of the full scope of the chimeric DNA or encoded proteins, by reference to knowledge in the art of the "structure, formula, chemical name, or physical

properties" of the DNA or the proteins. In the Board's words:

> [*1355] We are led by controlling precedent to understand that the full scope of novel chimeric DNA the parties claim is not described in their specifications under *35 U.S.C. § 112*, first paragraph, by reference to contemporary and/or prior knowledge in the art of the structure, formula, chemical name, or physical properties of many protein domains, and/or DNA sequences which encode many protein domains, which comprise single-chain proteins and/or DNA constructs made in accordance with the plans, schemes, and examples thereof the parties disclose.

Bd. op. at 4. As controlling precedent the Board cited *Regents of the University of California v. Eli Lilly & Co., 119 F.3d 1559 (Fed. Cir. 1997)*; [**14] *Fiers v. Revel, 984 F.2d 1164 (Fed. Cir. 1993)*; *Amgen, Inc. v. Chugai Pharmaceutical Co., 927 F.2d 1200 (Fed. Cir. 1991)*; and *Enzo Biochem, Inc. v. Gen-Probe, Inc., 296 F.3d 1316 (Fed. Cir. 2002)*. The Board summarized its holding as follows:

> Here, both Eshhar and Capon claim novel genetic material described in terms of the functional characteristics of the protein it encodes. Their specifications do not satisfy the written description requirement because persons having ordinary skill in the art would not have been able to visualize and recognize the identity of the claimed genetic material without considering additional knowledge in the art, performing additional experimentation, and testing to confirm results.

Bd. op. at 89.

DISCUSSION

Eshhar and Capon challenge both the Board's interpretation of precedent and the Board's ruling that their descriptions are inadequate. Both parties explain that their chimeric genes are produced by selecting and combining known heavy-and light-chain immune-related DNA segments, using known DNA-linking procedures. The specifications of both parties describe procedures for identifying [**15] and obtaining the desired immune-related DNA segments and linking them into the desired chimeric genes. Both parties point to their specific examples of chimeric DNA prepared using identified known procedures, along with citation to the scientific literature as to every step of the preparative method.

The parties presented expert witnesses who placed the invention in the context of prior knowledge and explained how the descriptive text would be understood by persons of skill in the field of the invention. The witnesses explained that the principle of forming chimeric genes from selected segments of DNA was known, as well as their methods of identifying, selecting, and combining the desired segments of DNA. Dr. Eshhar presented an expert statement wherein he explained that the prior art contains extensive knowledge of the nucleotide structure of the various immune-related segments of DNA; he stated that over 785 mouse antibody DNA light chains and 1,327 mouse antibody DNA heavy chains were known and published as early as 1991. Similarly Capon's expert Dr. Desiderio discussed the prior art, also citing scientific literature:

> The linker sequences disclosed in the *'221 patent* [**16] (col. 24, lines 4 and 43) used to artificially join a heavy and light chain nucleic acid sequence and permit functional association of the two ligand binding regions were published by 1990, as were the methods for obtaining the mature sequences of the desired heavy and light chains for constructing a SAb (Exhibit 47, Batra et al., J., Biol. Chem., 1990; Exhibit 48, Bird et al., Science, 1988; Exhibit 50, Huston et al., PNAS, [*1356] 1988; Exhibit 51, Chaudhary, PNAS, 1990, Exhibit 56, Morrison et al., Science, 1985; Exhibit 53, Sharon et al., Nature 1984).

Desiderio declaration at 4 P11.

Both parties stated that persons experienced in this field would readily know the structure of a chimeric gene made of a first segment of DNA encoding the single-chain variable region of an antibody, and a second segment of DNA encoding an endogenous protein. They testified that re-analysis to confirm these structures would not be needed in order to know the DNA structure of the chimeric gene, and that the Board's requirement that the specification must reproduce the "structure, formula, chemical name, or physical properties" of these DNA combinations had been overtaken by the state of the science. [**17] They stated that where the structure and properties of the DNA components were known, reanalysis was not required.

Eshhar's specification contains the nucleotide sequences of sixteen different receptor primers and four different scFv primers from which chimeric genes encod-

ing scFvR may be obtained, while Capon's specification cites literature sources of such information. Eshhar's specification shows the production of chimeric genes encoding scFvR using primers, as listed in Eshhar's Table I. Capon stated that natural genes are isolated and joined using conventional methods, such as the polymerase chain reaction or cloning by primer repair. Capon, like Eshhar, discussed various known procedures for identifying, obtaining, and linking DNA segments, accompanied by experimental examples. The Board did not dispute that persons in this field of science could determine the structure or formula of the linked DNA from the known structure or formula of the components.

The Board stated that "controlling precedent" required inclusion in the specification of the complete nucleotide sequence of "at least one" chimeric gene. Bd. op. at 4. The Board also objected that the claims were broader than [**18] the specific examples. Eshhar and Capon each responds by pointing to the scientific completeness and depth of their descriptive texts, as well as to their illustrative examples. The Board did not relate any of the claims, broad or narrow, to the examples, but invalidated all of the claims without analysis of their scope and the relation of claim scope to the details of the specifications.

Eshhar and Capon both argue that they have set forth an invention whose scope is fully and fairly described, for the nucleotide sequences of the DNA in chimeric combination is readily understood to contain the nucleotide sequences of the DNA components. Eshhar points to the general and specific description in his specification of known immune-related DNA segments, including the examples of their linking. Capon points similarly to his description of selecting DNA segments that are known to express immune-related proteins, and stresses the existing knowledge of these segments and their nucleotide sequences, as well as the known procedures for selecting and combining DNA segments, as cited in the specification.

Both parties argue that the Board misconstrued precedent, and that precedent does not establish [**19] a *per se* rule requiring nucleotide-by-nucleotide re-analysis when the structure of the component DNA segments is already known, or readily determined by known procedures.

The Statutory Requirement

The required content of the patent specification is set forth in *Section 112 of Title 35*:

> *§ 112* P1. The specification shall contain a written description of the invention, [*1357] and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same, and shall set forth the best mode contemplated by the inventor of carrying out his invention.

The "written description" requirement implements the principle that a patent must describe the technology that is sought to be patented; the requirement serves both to satisfy the inventor's obligation to disclose the technologic knowledge upon which the patent is based, and to demonstrate that the patentee was in possession of the invention that is claimed. See *Enzo Biochem, 296 F.3d at 1330* (the written description requirement "is the quid pro quo [**20] of the patent system; the public must receive meaningful disclosure in exchange for being excluded from practicing the invention for a limited period of time"); *Reiffin v. Microsoft Corp., 214 F.3d 1342, 1345-46 (Fed. Cir. 2000)* (the purpose of the written description requirement "is to ensure that the scope of the right to exclude ...does not overreach the scope of the inventor's contribution to the field of art as described in the patent specification"); *In re Barker, 559 F.2d 588, 592 n. 4 (CCPA 1977)* (the goal of the written description requirement is "to clearly convey the information that an applicant has invented the subject matter which is claimed"). The written description requirement thus satisfies the policy premises of the law, whereby the inventor's technical/scientific advance is added to the body of knowledge, as consideration for the grant of patent exclusivity.

The descriptive text needed to meet these requirements varies with the nature and scope of the invention at issue, and with the scientific and technologic knowledge already in existence. The law must be applied to each invention that enters the patent process, for each patented [**21] advance is novel in relation to the state of the science. Since the law is applied to each invention in view of the state of relevant knowledge, its application will vary with differences in the state of knowledge in the field and differences in the predictability of the science.

For the chimeric genes of the Capon and Eshhar inventions, the law must take cognizance of the scientific facts. The Board erred in refusing to consider the state of the scientific knowledge, as explained by both parties, and in declining to consider the separate scope of each of the claims. None of the cases to which the Board attributes the requirement of total DNA re-analysis, i.e., Regents v. Lilly, Fiers v. Revel, Amgen, or Enzo Biochem, require a re-description of what was already known. In *Lilly, 119 F.3d at 1567*, the cDNA for human insulin had

never been characterized. Similarly in *Fiers, 984 F.2d at 1171*, much of the DNA sought to be claimed was of unknown structure, whereby this court viewed the breadth of the claims as embracing a "wish" or research "plan." In *Amgen, 927 F.2d at 1206*, the court explained that a novel gene was [**22] not adequately characterized by its biological function alone because such a description would represent a mere "wish to know the identity" of the novel material. In *Enzo Biochem, 296 F.3d at 1326*, this court reaffirmed that deposit of a physical sample may replace words when description is beyond present scientific capability. In *Amgen Inc. v. Hoechst Marion Roussel, Inc., 314 F.3d 1313, 1332 (Fed. Cir. 2003)* the court explained further that the written description requirement may be satisfied "if in the knowledge of the art the disclosed function is sufficiently correlated to a particular, known structure." These evolving principles were applied in *Noelle* [*1358] *v. Lederman, 355 F.3d 1343, 1349 (Fed. Cir. 2004)*, where the court affirmed that the human antibody there at issue was not adequately described by the structure and function of the mouse antigen; and in *University of Rochester v. G.D. Searle & Co., 358 F.3d 916, 925-26 (Fed. Cir. 2004)*, where the court affirmed that the description of the COX-2 enzyme did not serve to describe unknown compounds capable of selectively inhibiting the enzyme.

The "written description" [**23] requirement must be applied in the context of the particular invention and the state of the knowledge. The Board's rule that the nucleotide sequences of the chimeric genes must be fully presented, although the nucleotide sequences of the component DNA are known, is an inappropriate generalization. When the prior art includes the nucleotide information, precedent does not set a *per se* rule that the information must be determined afresh. Both parties state that a person experienced in the field of this invention would know that these known DNA segments would retain their DNA sequences when linked by known methods. Both parties explain that their invention is not in discovering which DNA segments are related to the immune response, for that is in the prior art, but in the novel combination of the DNA segments to achieve a novel result.

The "written description" requirement states that the patentee must describe the invention; it does not state that every invention must be described in the same way. As each field evolves, the balance also evolves between what is known and what is added by each inventive contribution. Both Eshhar and Capon explain that this invention does not concern [**24] the discovery of gene function or structure, as in Lilly. The chimeric genes here at issue are prepared from known DNA sequences of known function. The Board's requirement that these sequences must be analyzed and reported in the specification does not add descriptive substance. The Board erred in holding that the specifications do not meet the written description requirement because they do not reiterate the structure or formula or chemical name for the nucleotide sequences of the claimed chimeric genes.

Claim Scope

There remains the question of whether the specifications adequately support the breadth of all of the claims that are presented. The Director argues that it cannot be known whether all of the permutations and combinations covered by the claims will be effective for the intended purpose, and that the claims are too broad because they may include inoperative species. The inventors say that they have provided an adequate description and exemplification of their invention as would be understood by persons in the field of the invention. They state that biological properties typically vary, and that their specifications provide for evaluation of the effectiveness [**25] of their chimeric combinations.

It is well recognized that in the "unpredictable" fields of science, it is appropriate to recognize the variability in the science in determining the scope of the coverage to which the inventor is entitled. Such a decision usually focuses on the exemplification in the specification. See, e.g., *Enzo Biochem, 296 F.3d at 1327-28* (remanding for district court to determine "whether the disclosure provided by the three deposits in this case, coupled with the skill of the art, describes the genera of claims 1-3 and 5"); *Lilly, 119 F.3d at 1569* (genus not described where "a representative number of cDNAs, defined by nucleotide sequence, falling within the scope of the genus" had not been provided); *In re Gosteli, 872 F.2d 1008, 1012 (Fed. Cir. 1989)* (two chemical compounds were insufficient [*1359] description of subgenus); *In re Smith, 59 C.C.P.A. 1025, 458 F.2d 1389, 1394-95 (CCPA 1972)* (disclosure of genus and one species was not sufficient description of intermediate subgenus); *In re Grimme, 47 C.C.P.A. 785, 274 F.2d 949, 952, 1960 Dec. Comm'r Pat. 123 (CCPA 1960)* (disclosure of single example and [**26] statement of scope sufficient disclosure of subgenus).

Precedent illustrates that the determination of what is needed to support generic claims to biological subject matter depends on a variety of factors, such as the existing knowledge in the particular field, the extent and content of the prior art, the maturity of the science or technology, the predictability of the aspect at issue, and other considerations appropriate to the subject matter. See, e.g., *In re Wallach, 378 F.3d 1330, 1333-34 (Fed. Cir. 2004)* (an amino acid sequence supports "the entire genus of DNA sequences" that can encode the amino acid sequence because "the state of the art has developed" such that it is

a routine matter to convert one to the other); *University of Rochester, 358 F.3d at 925* (considering whether the patent disclosed the compounds necessary to practice the claimed method, given the state of technology); *Singh v. Brake, 317 F.3d 1334, 1343, 48 Fed. Appx. 766 (Fed. Cir. 2002)* (affirming adequacy of disclosure by distinguishing precedent in which the selection of a particular species within the claimed genus had involved "highly unpredictable results").

It [**27] is not necessary that every permutation within a generally operable invention be effective in order for an inventor to obtain a generic claim, provided that the effect is sufficiently demonstrated to characterize a generic invention. See *In re Angstadt, 537 F.2d 498, 504 (CCPA 1976)* ("The examples, both operative and inoperative, are the best guidance this art permits, as far as we can conclude from the record"). While the Board is correct that a generic invention requires adequate support, the sufficiency of the support must be determined in the particular case. Both Eshhar and Capon present not only general teachings of how to select and recombine the DNA, but also specific examples of the production of specified chimeric genes. For example, Eshhar points out that in Example 1 of his specification the FcR . chain was used, which chain was amplified from a human cDNA clone, using the procedure of Kuster, H. et al., J. Biol. Chem., 265:6448-6451 (1990), which is cited in the specification and reports the complete sequence of the FcRy chain. Eshhar's Example 1 also explains the source of the genes that provide the heavy and light chains of the single chain antibody, [**28] citing the PhD thesis of Gideon Gross, a co-inventor, which cites a reference providing the complete sequence of the Sp6 light chain gene used to construct the single-chain antibody. Eshhar states that the structure of the Sp6 heavy chain antibody was well known to those of skill in the art and readily accessible on the internet in a database as entry EMBL: MMSP6718. Example 5 at page 54 of the Eshhar specification cites Ravetch et al., J. Exp. Med., 170:481-497 (1989) for the method of producing the CD16 a DNA clone that was PCR amplified; this reference published the complete DNA sequence of the CD16 a chain, as discussed in paragraph 43 of the Eshhar Declaration. Example 3 of the Eshhar specification uses the DNA of the monoclonal anti-HER2 antibody and states that the N29 hybridoma that produces this antibody was deposited with the Collection Nationale de Cultures de Microorganismes, Institut Pasteur, Paris, on August 19, 1992, under Deposit No. CNCM I-1262. It is incorrect to criticize the methods, examples, and referenced prior art of the Eshhar specification as but "a few PCR primers and probes," as does the Director's brief.

[*1360] Capon's Example 3 provides a detailed description [**29] of the creation and expression of single chain antibody fused with T-cell receptor zeta chain, referring to published vectors and procedures. Capon, like Eshhar, describes gene segments and their ligation to form chimeric genes. Although Capon includes fewer specific examples in his specification than does Eshhar, both parties used standard systems of description and identification, as well as known procedures for selecting, isolating, and linking known DNA segments. Indeed, the Board's repeated observation that the full scope of all of the claims appears to be "enabled" cannot be reconciled with the Board's objection that only a "general plan" to combine unidentified DNA is presented. See *In re Wands, 858 F.2d 731, 736-37 (Fed. Cir. 1988)* (experimentation to practice invention must not be "undue" for invention to be considered enabled).

The PTO points out that for biochemical processes relating to gene modification, protein expression, and immune response, success is not assured. However, generic inventions are not thereby invalid. Precedent distinguishes among generic inventions that are adequately supported, those that are merely a "wish" or "plan," the words of [**30] *Fiers v. Revel, 984 F.2d at 1171*, and those in between, as illustrated by *Noelle v. Lederman, 355 F.3d at 1350*; the facts of the specific case must be evaluated. The Board did not discuss the generic concept that both Capon and Eshhar described — the concept of selecting and combining a gene sequence encoding the variable domain of an antibody and a sequence encoding a lymphocyte activation protein, into a single DNA sequence which, upon expression, allows for immune responses that do not occur in nature. The record does not show this concept to be in the prior art, and includes experimental verification as well as potential variability in the concept.

Whether the inventors demonstrated sufficient generality to support the scope of some or all of their claims, must be determined claim by claim. The Board did not discuss the evidence with respect to the generality of the invention and the significance of the specific examples, instead simply rejecting all the claims for lack of a complete chimeric DNA sequence. As we have discussed, that reasoning is inapt for this case. The Board's position that the patents at issue were merely an "invitation to [**31] experiment" did not distinguish among the parties' broad and narrow claims, and further concerns enablement more than written description. See *Adang v. Fischhoff, 286 F.3d 1346, 1355 (Fed. Cir. 2002)* (enablement involves assessment of whether one of skill in the art could make and use the invention without undue experimentation); *In re Wright, 999 F.2d 1557, 1561 (Fed. Cir. 1993)* (same). Although the legal criteria of enablement and written description are related and are often met by the same disclosure, they serve discrete legal requirements.

The predictability or unpredictability of the science is relevant to deciding how much experimental support is required to adequately describe the scope of an invention. Our predecessor court summarized in *In re Storrs, 44 C.C.P.A. 981, 245 F.2d 474, 478, 1957 Dec. Comm'r Pat. 361 (CCPA 1957)* that "it must be borne in mind that, while it is necessary that an applicant for a patent give to the public a complete and adequate disclosure in return for the patent grant, the certainty required of the disclosure is not greater than that which is reasonable, having due regard to the subject matter involved." This aspect may [**32] warrant exploration on remand.

In summary, the Board erred in ruling that *§ 112* imposes a *per se* rule requiring recitation in the specification of the nucleotide [*1361] sequence of claimed DNA, when that sequence is already known in the field. However, the Board did not explore the support for each of the claims of both parties, in view of the specific examples and general teachings in the specifications and the known science, with application of precedent guiding review of the scope of claims.

We remand for appropriate further proceedings.

VACATED AND REMANDED

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Molecular Cloning of cDNA Encoding the p70 (Ku) Lupus Autoantigen*

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The Ku (p70/p80) autoantigen consists of two phosphoproteins of molecular mass ~70,000 and 80,000 forming a macromolecular complex that binds DNA. Autoantibodies from a patient with systemic lupus erythematosus were used to isolate cDNA clones encoding the human ~70-kDa Ku antigen (p70) from a λgt11 expression library. The deduced amino acid sequence of p70 consisted of 609 amino acid residues and was confirmed by partial amino acid sequencing. The protein contains two acidic domains of 61 residues (31% Glu + Asp) and 19 residues (53% Glu + Asp) that are similar in size and charge to those found in a number of proteins involved in transcriptional activation. The 61-residue acidic region is rich in serine, raising the possibility that its charge might be modulated by phosphorylation. The predicted amino acid sequence also contains two regions with periodic repeats of either leucine alone, or leucine alternating with serine every seventh position. The latter repeat displays sequence and secondary structural similarities with the "leucine zipper" regions of the c-*myc* and v-*myc* oncogene products. The p70 antigen does not appear to have extensive sequence homology with the 80-kDa Ku autoantigen based on analysis of RNA blots and immunological criteria. A major antigenic determinant or determinants recognized by human autoantibodies is located near a leucine repeat on the carboxyl-terminal 190 amino acid residues of p70.

The p70/p80 autoantigen consists of two proteins of molecular mass ~70,000 and ~80,000 daltons that dimerize to form a 10 S DNA-binding complex (1). Exchange of immunological reagents has established that the p70/p80 antigen (1, 2), Ku antigen (3-5), Ki antigen (6), as well as a 86-70-kDa protein complex (7, 8)[1] are identical. The p70/p80 complex binds to the ends of double-stranded DNA (4) in a cell cycle-dependent manner, being associated with chromosomes of interphase cells, followed by complete dissociation from the condensing chromosomes in early prophase (2). Both p70 and p80 have been found to contain phosphoserine residues (8). The function of the antigen is unknown, but a role in DNA repair or transposition has been proposed (4, 5). Certain individuals with systemic lupus erythematosus (SLE)[2] and related disorders produce extremely large amounts of autoantibodies to p70 and p80 (1, 3, 6). We have used autoantibodies from the serum of an individual with SLE to isolate cDNA clones encoding p70, the protein that is thought to mediate binding of the Ku (p70/p80) complex to DNA (5). Analysis of the predicted amino acid sequence of p70 suggests structural similarities with other DNA-binding proteins. The amino acid sequence should be useful for examining the function of the Ku (p70/p80) complex, as well as the causes of autoimmunity to this antigen.

MATERIALS AND METHODS

Isolation of cDNA Clones—Human autoantibodies to the Ku (p70/p80) antigen from a patient (CK) with SLE were used to screen a human hepatoma λgt11 cDNA library, provided by M. Mueckler (Whitehead Institute, Cambridge, MA), using established protocols (9-11). Recombinant phage were plated on lawns of *Escherichia coli* Y1090 and overlaid with nitrocellulose filters (Schleicher & Schuell, BA85) impregnated with isopropylthiogalactoside (Sigma). Positive plaques were detected by incubating in blocking solution (150 mM NaCl, 50 mM Tris, pH 7.5, 1% bovine hemoglobin, 0.02% NaN_3) for 1 h at 22 °C, followed by CK serum (1:5000 in blocking solution, which was preadsorbed with bacterial lysate) (11) for 8 h at 4 °C, and ^{125}I-protein A (Du Pont-New England Nuclear; 10^6 dpm/ml) for 3 h at 22 °C. Three cDNA clones were obtained, the longest of which (~2.0 kb) was used to screen the same library by nucleic acid hybridization (12). Probes were labeled with [α^{32}P]dCTP by random priming (13) using Klenow fragment (Amersham Corp.). In addition, a 27-bp oligonucleotide 5′-CTTCCTCTGCTTCTTCATCGCCCTCGG-3′ complementary to the 5′ end of the of the 2.0-kb clone was synthesized (Applied Biosystems 380A DNA synthesizer), ^{32}P end-labeled with polynucleotide kinase (14) and used to rescreen the library (15).

Production of p70 Fusion Proteins—λgt11 clones 70.5, 70.34, and 70.77 were used to lysogenize *E. coli* Y1089, and fusion proteins were isolated as described (11). *E. coli* lysates containing the fusion proteins were analyzed on 8% SDS-polyacrylamide gels, and stained with Coomassie Brilliant Blue R250 (16).

Immunoblotting of the fusion proteins was performed as described (17). Blots were incubated in blocking solution for >1 h, followed by CK serum (1:250 dilution), or by the same dilution of CK serum plus an irrelevant autoimmune serum (patient JK) at a dilution of 1:250 for 3 h at 22 °C. After washing three times for 30 min, the blots were incubated with alkaline phosphatase-conjugated goat anti-human IgG antibodies (1:1500 dilution, from Tago, Burlingame, CA) for 3 h at 22 °C. Antibodies specific for the fusion proteins were purified by elution from the nitrocellulose blots (18) and used to probe immunoblots of K562 nuclear extract (2) followed by detection with ^{125}I-protein A as described above.

DNA Sequence Analysis—Restriction fragments of the phage cDNA inserts were subcloned into pUC 19, subsequently into

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) J04611.

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[1] M. Yaneva, personal communication.

[2] The abbreviations used are: SLE, systemic lupus erythematosus; kb, kilobase(s); bp, base pair(s); SDS, sodium dodecyl sulfate.

M13mp18 or M13mp19 (19), and sequenced from both strands by the dideoxy chain termination method (20). The rapid deletion subcloning technique of Dale *et al.* (21) was utilized to generate a sequential series of overlapping clones for sequencing. Oligonucleotides were synthesized and used without further purification (22) as primers for sequencing certain large fragments. Modified T7 DNA polymerase (Sequenase, United States Biochemical Corp., Cleveland, OH) using dITP in place of dGTP (23) was used for dideoxy sequencing of DNA regions not adequately resolved with Klenow fragment.

Computer Sequence Analysis—Sequences were assembled and analyzed by computer programs provided by the BIONET National Computer Resource for Molecular Biology. The translated amino acid sequence of p70 was compared to sequences in the National Biomedical Research Foundation Protein Identification Resource (PIR) using the algorithms of Lipman and Pearson (24, 25). Statistical significance of alignments was evaluated using the RDF program (24).

Protein Sequencing—Ku (p70/p80) antigen was purified from ~3.5 × 10^9 K562 cells as described.[3] Protein A-Sepharose beads were coated with monoclonal antibody 162 (1) at 4 °C for 8 h, washed three times with 150 mM NaCl, 10 mM Tris, pH 8.0, 1 mM EDTA, 0.5% Nonidet P-40, 1 mg/ml ovalbumin, 0.02% NaN_3, and added to an extract of K562 cells (in 150 mM NaCl, 50 mM Tris, pH 7.5, 1 mM EDTA, 0.5 mM phenylmethylsulfonyl fluoride) for 3 h at 4 °C. The beads were washed three times with 150 mM NaCl, 50 mM Tris, pH 7.5, 2 mM EDTA, 0.25 M sucrose, 2.5% Triton X-100, 0.5% SDS, then three times with 150 mM NaCl, 50 mM Tris, pH 7.5, 2 mM EDTA, and heated to 100 °C for 3 min in SDS sample buffer (16) before resolving on 10% SDS-polyacrylamide gels. The gels were stained with Coomassie Brilliant Blue R-250, and gel slices containing p70 were excised. The protein was electroeluted from the gel exactly as described by Hunkapiller *et al.* (27).

Electroeluted p70 was cleaved with chymotrypsin (Worthington) as follows: approximately 7 μg of p70 in 60 μl of 0.125 M Tris, pH 6.8, 0.5% SDS, 10% glycerol, 0.0001% bromphenol blue was heated to 100 °C for 3 min before adding chymotrypsin to a final concentration of 17 μg/ml. The sample was incubated for 30 min at 37 °C; digestion was terminated by the addition of SDS to 2.5% and dithiothreitol to 0.1 M. The sample was then heated to 55 °C for 10 min and loaded onto a 12.5% SDS-polyacrylamide gel.

After electrophoresis, intact p70 and chymotryptic peptides were transferred to polyvinylidene difluoride membrane (Immobilon, Whatman, Clifton, NJ) (28). After visualization by Coomassie Blue staining, p70 and p70 peptides of ~29, 22, and 16 kDa were excised from the blot and subjected to automated Edman degradation with the Applied Biosystems model 470A gas-phase sequencer. The phenylhydantoin amino acid derivatives were identified and quantitated using a Hewlett Packard 1084 HPLC system.

RNA Blot Analysis—K562 poly(A)$^+$ RNA (29, 30) was separated on 0.8% agarose gels containing 2.2 M formaldehyde (14), transferred to nitrocellulose, and baked for 90 min at 80 °C (31). DNA probes were labeled by random priming (13) as described above. RNA blots were prehybridized for 6–12 h at 42 °C in 5 × SSPE (1 × SSPE = 0.15 M NaCl, 10 mM sodium phosphate, pH 7.4, 1 mM EDTA), 10 × Denhardt's solution (1 × = 0.02% Ficoll, 0.02% polyvinylpyrrolidone, 0.02% bovine serum albumin), 50% formamide, 0.4 mg/ml denatured sonicated salmon sperm DNA, 0.1% SDS before hybridizing for 30 h in the same solution containing probe at 10^6 dpm/ml at 42 °C. The blots were washed at 65 °C with 2 × SSC (1 × SSC = 0.15 M NaCl, 15 mM sodium citrate, pH 7.4), 0.1% SDS (three times, 10 min each) followed by 0.3 × SSC, 0.1% SDS (three times, 45 min each), and exposed to X-ray film (XAR-5, Kodak, Rochester, NY) with Lightening Plus intensifying screens (Du Pont-New England Nuclear).

RESULTS

Isolation of cDNA Clones Encoding p70 Epitopes—A λgt11 expression library was screened with serum from a patient (CK) with high titer anti-Ku (p70/p80) antibodies. This serum contains anti-Ku (p70/p80) antibodies at a titer of approximately 1:3 × 10^6, along with low levels (1:1000 titer or less) of anti-RNP and anti-Sm antibodies (32). At the 1:5000 dilution used for screening, the serum was essentially monospecific for p70. Screening the λgt11 library with this serum yielded three positive plaques, designated clones 70.5, 70.34, and 70.77, respectively (Fig. 1). After plaque purification, *Eco*RI digestion of purified phage DNA demonstrated insert DNA fragments of approximately 1600 and 350 bp (clone 70.5), 900 bp (clone 70.34), and 700 bp (clone 70.77). On Southern blots, insert DNA from clone 70.77 hybridized with insert DNA from clone 70.34, and with the ~1600-bp fragment from clone 70.5 (not shown). DNA sequence analysis (see below) confirmed that the three clones contained fragments of the same gene.

Nucleic acid hybridization screening yielded additional λgt11 clones hybridizing with both the clone 70.77 insert and with the ~350-bp fragment of clone 70.5. Restriction mapping suggested that two of these clones, designated 70.30 and 70.45 (Fig. 1) contained additional DNA sequences not contained by clone 70.5. Screening with the 5′-oligonucleotide failed to yield clones with longer inserts.

E. coli lysogenic for λgt11 clones 70.34 and 70.77 produced fusion proteins of ~145 and ~140 kDa, respectively, after induction with isopropylthiogalactoside (Fig. 2). *E. coli* lysogenic for clone 70.5 produced only trace quantities of fusion protein (not shown). Autoantibodies from CK serum were affinity purified on nitrocellulose-bound 70.34 or 70.77 fusion proteins and used to probe immunoblots of total nuclear proteins (Fig. 3). The affinity-purified anti-70.34 and anti-70.77 antibodies specifically bound to p70 on immunoblots of total nuclear proteins, while autoantibodies in the original CK serum bound to both p70 and p80 (Fig. 3*A*). Addition of JK autoimmune serum to CK serum resulted in binding to additional proteins on immunoblots (Fig. 3*B*, *CK+JK*). The contaminating JK autoantibodies were removed by affinity purification on 70.34 and 70.77 (Fig. 3*B*), demonstrating the specificity of binding to the fusion proteins.

DNA Sequence—The nucleotide sequence of cDNAs from clones 70.5, 70.34, 70.77, 70.30, and 70.45 was determined from both strands using the sequencing strategy shown in Fig. 1. The nucleotide sequence (Fig. 4) contains a single open reading frame of 1,827 bp (from nucleotide 34 to 1,860), coding for 609 amino acids. The predicted molecular mass of the encoded p70 protein is 69,851, in close agreement with the apparent molecular mass of 70,000 estimated by SDS-polyacrylamide gel electrophoresis (1). The open reading frame is preceded by a 5′-untranslated region of 33 bp, and followed by a 3′-untranslated region of 294 bp terminating with a

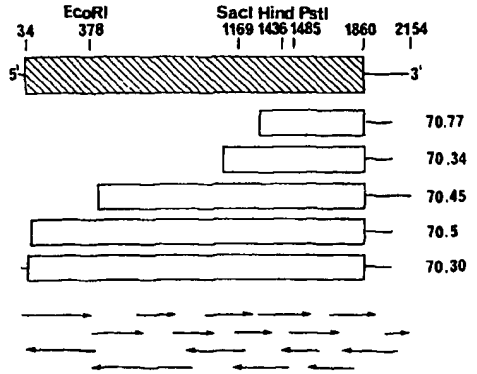

FIG. 1. p70 partial restriction map, clones, and sequencing strategy. The coding region (bases 34–1860) is shown as a *hatched box* in the partial restriction map (*top*). The individual cDNA clones obtained by screening with antibody probes are labeled *70.77* (bases 1286–2027), *70.34* (bases 1112–2025), and *70.5* (bases 44–2021). Additional cDNA clones obtained by nucleic acid hybridization are labeled *70.45* and *70.30*. The sequencing strategy is indicated by *arrows* at the *bottom*.

[3] W. H. Reeves, Z. M. Sthoeger, and R. G. Lahita, manuscript submitted for publication.

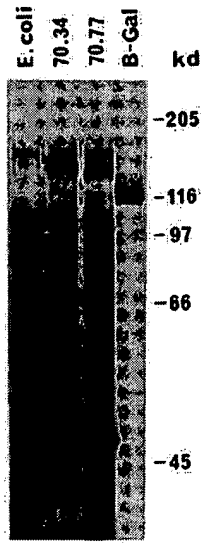

FIG. 2. **SDS-polyacrylamide gel of fusion proteins obtained from *E. coli* Y1089 lysogenized by λgt11 clones.** *E. coli* were solubilized in SDS sample buffer, and proteins were resolved on an 8% SDS-polyacrylamide gel followed by Coomassie Blue staining. Lanes show *E. coli* Y1089 lysate, and lysates of *E. coli* Y1089 lysogenized by clones 70.34 and 70.77. The last lane shows purified β-galactosidase (Sigma) for comparison. Positions of molecular mass markers are indicated on the *right*. *kd*, kilodaltons.

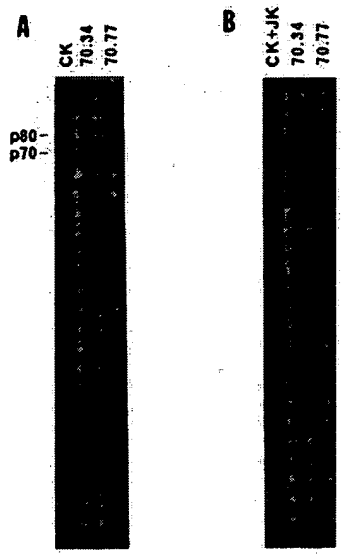

FIG. 3. **Immunoblots of antibodies affinity-purified from blots of fusion proteins.** *A*, immunoblots of K562 nuclear extract using CK serum (1:500) or CK antibodies (initial serum dilution 1:250) affinity-purified from 70.34 or 70.77 fusion proteins, respectively. On immunoblots of total nuclear extract, CK serum reacted with both p70 and p80, while the affinity-purified antibodies were specific for p70. *B*, immunoblots of K562 nuclear extract using CK plus JK sera (both at 1:500 dilution) or CK plus JK sera (initially each at 1:250) affinity-purified from 70.34 or 70.77 fusion proteins, respectively.

AATAAA sequence followed by a 68-bp poly(A) sequence. Two clones (70.5 and 70.44) had a cytidine at position 300, while two others (70.30 and 70.26) had a thymidine. The substitution does not change the predicted amino acid sequence and may represent allelic variation.

The sequence AACATG (nucleotides 31–36) is a potential ribosome binding site (33) which may encode the initiator methionine as indicated in Fig. 4. However, this prediction could not be confirmed by amino acid sequencing because the amino terminus of p70 was blocked.

Partial Amino Acid Sequence of p70—Since the amino-terminal sequence of p70 was unobtainable, the protein was cleaved with chymotrypsin and partial amino acid sequences of peptides of molecular mass ~29, 27, and 16 kDa were determined. The amino acid sequences of the three peptides match the predicted amino acid sequence as shown in Fig. 4 (*single letter code*), confirming the identity of the cDNA clone.

RNA Blot Analysis—Probes consisting of the 3′ ~1640 bp and 5′ ~340 bp of clone 70.5 each hybridized with a single mRNA species of ~2.4 kb (Fig. 5, *probes A* and *B*, respectively). Thus, although the entire coding sequence has probably been determined, the sequence of the 5′-untranslated region is likely incomplete.

p70 Has a Cluster of Acidic Amino Acids and Periodic Repeats of Leucine or Leucine and Serine Residues—Examination of the predicted amino acid sequence of p70 revealed the existence of a high concentration of negatively charged residues near the amino terminus. The first 61 amino acids consist of 31% glutamic acid + aspartic acid, with a 19-amino acid region (residues 10–28, *underlined* in Fig. 4) consisting of 58% Glu + Asp. In addition, the amino-terminal 81 amino acids contains 13 serine residues (16%). A shorter acidic domain is present from residues 328–340 (7/13 residues or 53% Glu + Asp, *underlined* in Fig. 4).

Comparison of the amino acid sequence with known sequences in the National Biomedical Research Foundation Protein Identification Resource database revealed a possibly significant similarity with the v-*myc* oncogene product (Fig. 6). A region of p70 from amino acid 187 to 248 (62 residues) was 27% identical with a region of the v-*myc* oncogene protein from amino acid 361 to 422, and displayed weaker similarity with the c-*myc* protein. Statistical analysis of this alignment using the RDF program (24) gave an initial score of 62 (z = 9.59 S.D.) the aligned score of 62 (z = 5.62 S.D.). This region of both v-*myc* and c-*myc* contains a "leucine zipper" domain characterized by the periodic repetition of leucine residues every seventh position in an α-helical region (34). The p70 sequence has identical periodicity, but instead of having leucine residues at every seventh position, has leucine alternating with serine (Figs. 4, 6, and 7, indicated by *). Secondary structure predictions for p70, v-*myc*, and c-*myc* in this region are suggestive of α-helix formation (Fig. 7). Immediately adjacent to this region (toward the carboxyl terminus) is a 22-amino acid region containing 50% basic residues (Fig. 7, indicated by *x*), as appears in other proteins with leucine repeats (34). Another possible leucine repeat in p70 occurs from amino acids 483 to 511 (Fig. 4, residues at seventh positions indicated by *), but contains a proline residue (residue 500) that might destabilize a region of α-helix.

DISCUSSION

The Ku (p70/p80) antigen is recognized by autoantibodies in sera of certain patients with SLE (1) and other (3) collagen vascular diseases. The function of this antigen is not known, but previous studies have shown that the p70 and p80 proteins form a complex (1, 6, 7) that binds to DNA (1, 4, 5, 7). Binding to DNA may be mediated by p70 (5) and also be specific for ends of double-stranded DNA, suggesting a possible role in DNA repair or transposition (4).

These previous studies suggest that the p70 protein contains a region, or regions, mediating binding to DNA and to p80. As a first step to defining these regions, we have cloned and sequenced cDNA encoding p70. The translated amino acid sequence consists of 609 amino acids (Fig. 4). However, the

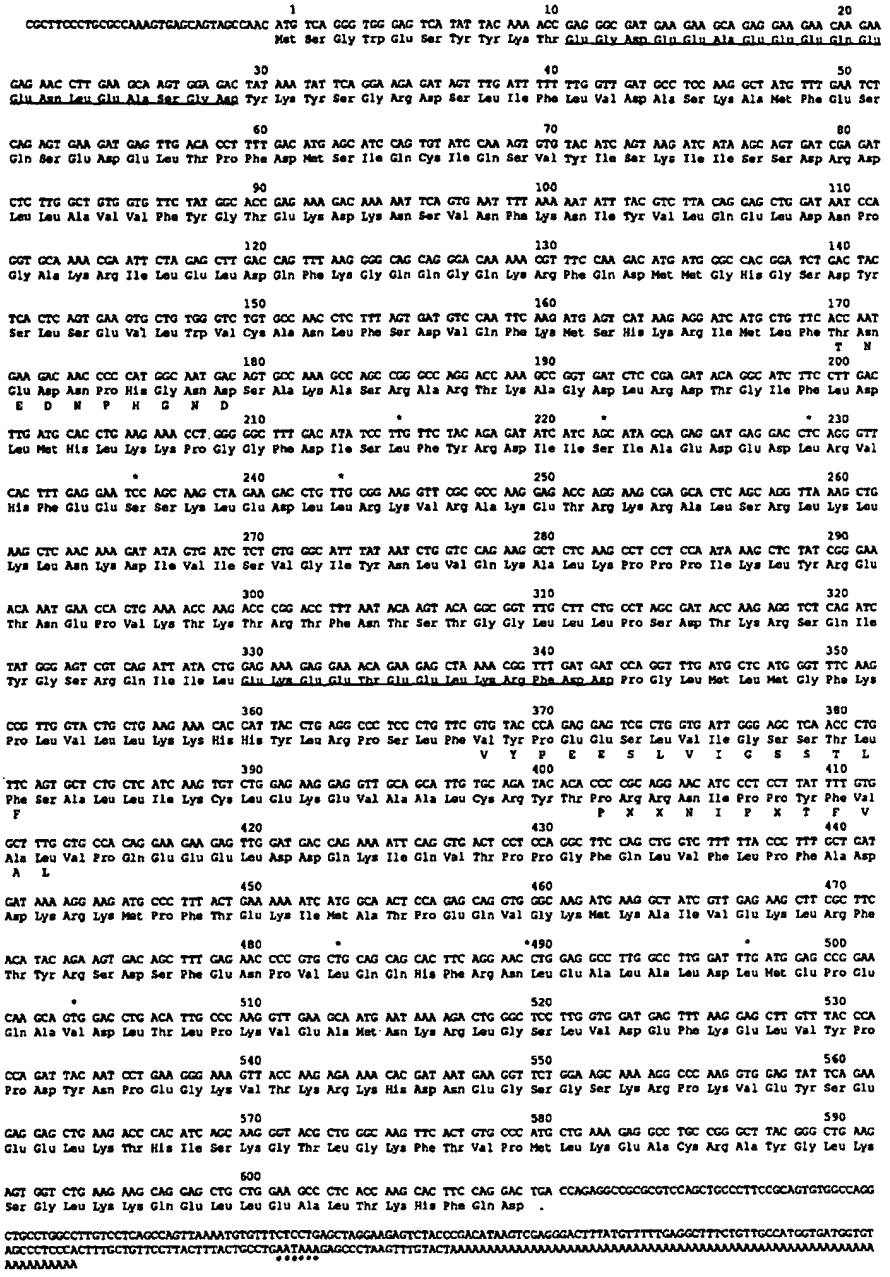

FIG. 4. **Nucleotide and translated amino acid sequence of p70.** DNA sequence is shown *above*, and predicted amino acid sequence *below* in *three-letter code*. Numbering corresponds to the predicted amino acid sequence. Amino acid sequences determined by automated Edmann degradation are indicated by *one-letter code* beneath the predicted amino acid sequence. Anionic domains of the translated protein (residues 11–29 and 330–342) are *underlined*. Periodic repeats of leucine and/or serine residues are indicated by *. A potential polyadenylation signal (AATAAA) is indicated (.....).

predicted initial methionine may be cleaved *in vivo*, since it is followed by serine, a residue that promotes removal of amino-terminal methionine residues by an amino-terminal methionine aminopeptidase (35). In addition, the amino terminus of p70 appears to be blocked. Acetylated methionine residues are generally not followed by serine (35, 36), while an amino-terminal serine residue is frequently acetylated (37), providing further indirect evidence that the amino-terminal residue *in vivo* may be serine rather than methionine.

Analysis of the predicted p70 amino acid sequence demonstrated two regions of possible α-helical secondary structure (Fig. 7) containing periodic repeats of either leucine and serine (residues 215–243) or leucine alone (residues 483–504) (Figs. 4 and 6). The Leu-Ser repeat region of p70 displays a possibly significant sequence similarity with a region of the v-*myc* and c-*myc* proteins that is essential for transformation (38), and which contains a leucine repeat with identical periodicity. While the functional significance of this similarity is difficult to assess at present, it is notable that two cellular differentiation factors, the MyoD1 protein (39) and the T4 achaetescute protein of *Drosophila* (40), also display comparable similarities with this region of *myc*.

The Leu- and Leu-Ser repeat regions of p70 are similar to leucine repeat regions found in a number of oncogene products and transcription factors (34). Many of these proteins contain a region rich in basic amino acids immediately adjacent to the leucine repeat, The Leu-Ser repeat of p70 is adjacent to a strongly basic region (Fig. 7) and the leucine repeat to a less strongly basic region (residues 461–482). In the model proposed by Landschulz *et al.* (34), the periodic repeat of leucine residues is thought to interdigitate with a similar domain of a second protein, juxtaposing the basic amino acids of the two proteins in a manner suitable for sequence-specific recognition of DNA. It remains to be determined whether either the

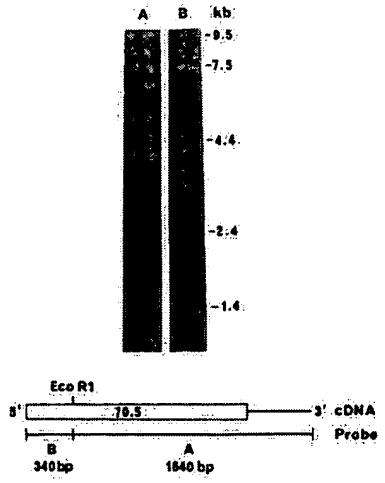

FIG. 5. **RNA blots of K562 poly(A)$^+$.** Poly(A)$^+$ RNA (13.2 μg/lane) was analyzed on a 1% agarose/formaldehyde gel and transferred to nitrocellulose. Blots were baked, prehybridized, and hybridized with ^{32}P-labeled *Eco*RI fragments of clone 70.5: *A* = ~1640 bp 3′ fragment; *B* = ~340 bp 5′ fragment. Both fragments hybridized with a RNA species of ~2.4 kb. Positions of RNA standards (Bethesda Research Laboratories, Gaithersburg, MD) are indicated.

```
                                                  *   *   *   *   *
p70     RTKAGDLRDTGIFLDLMHLKKPGGFDISLFYRDIISIAEDEDLRVHFEESSKLEDLLRKVRA
        :.. .. ...  ... ::::... .::  .  ::::.:.:: . :. .. . :::. :
v-myc   RDQIPEVANNEKAPKVVILKKATEYVLSLQSDEHKLIAEKEQLRRRREQLKHNLEQLRNSRA
        :.. .: ...  ... ::::. . .:.  .  :: :: . :. .. . :::. :
c-myc   RDQIPELENNEKAPKVVILKKATAYILSVQAEEQKLISEEDLLRKRREQLKHKLEQLRNSCA
```

FIG. 6. **Amino acid sequence similarity between p70, v-*myc*, and c-*myc*.** The deduced amino acid sequence of p70 (residues 187–248) was aligned to maximize similarity with the amino acid sequences of v-*myc* (avian myelocytomatosis virus) (49), residues 361–422, and human c-*myc* (50), residues 399–460. This region of similarity coincides with the proposed "leucine zipper" domain of the *myc* proteins (34). Positions of the periodic repeats of leucine and serine (p70) or leucine alone (v-*myc* and c-*myc*) are indicated by *.

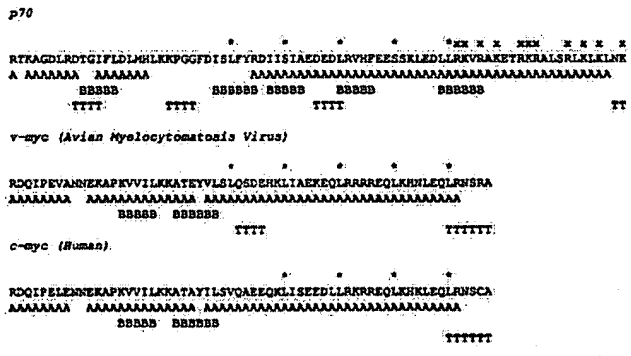

FIG. 7. **Predicted secondary structures of similar regions of p70; v-*myc*, and c-*myc*.** *A* denotes helix-permissive structure, *B* denotes β-sheet, and *T* denotes turn, as predicted by the program of Chou and Fasman (26). Positions of periodic repeats of leucine and serine (p70) or leucine alone (v-*myc* and c-*myc*) are indicated (*). Basic residues in a 22-amino acid region immediately following the leucine-serine repeat of p70 are indicated by *x*.

leucine repeat or the Leu-Ser repeat can participate in the formation of this hypothetical structure. In particular, we cannot be certain that a polar amino acid such as serine would be compatible with the interdigitation postulated by the Landschulz model. The sequence similarity of p70 with the leucine zipper region of *myc*, the α-helical secondary structure predicted for this region (Fig. 7), and the adjacent 22-residue basic domain may provide indirect evidence supporting this possibility. Clearly, however, further experimental evidence will be necessary to assess the functional significance, if any, of this region. If either of these repeats is involved in the formation of a leucine zipper, then the Landschulz model would predict the existence of a similar region(s) in the p80 protein. This prediction will be readily testable when the sequence of p80 is available.

The predicted amino acid sequence of p70 also contains two regions rich in acidic residues (61 residues, 31% Glu + Asp, and 19 residues, 58% Glu + Asp, see Fig. 4). These acidic regions are comparable in length and charge to the acidic domains found in GCN4 (60 amino acids, 30% Glu + Asp) (41), and GAL4 (29 residues, 31% Glu + Asp, and 20 residues, 35% Glu + Asp) (42) that are thought to play a critical role in transcriptional activation (41–43). In addition, the high frequency of serine residues in the 61-amino acid acidic domain raises the possibility that the negative charge of this region might be increased by phosphorylation. Since the acidity of an "acid blob" appears to correlate with its transcriptional potency (44), phosphorylation of this region, if it occurs, might have functional significance. Thus, the structure of p70 resembles that of GCN4 and *myc* proteins not only in containing one or more possible leucine zipper domains (34, 41), but also in containing an anionic region (41, 45). Based on the existence of both a possible DNA-binding domain(s) and a potential transcriptional activator domain (43), it is tempting to speculate that p70 might have a role in transcription. Alternatively, the structure of p70 might be consistent with a role in DNA repair (4) or replication. These possibilities are not mutually exclusive, since recent studies indicate that certain transcriptional activators may be components of eukaryotic origins of DNA replication (46, 47).

The present studies demonstrate the existence of a major autoantigenic epitope or epitopes on the carboxyl-terminal 190 amino acids of p70 (Fig. 3, *70.77*), a region containing the leucine repeat region of p70 (Fig. 4). We have previously found that autoantibodies in certain autoimmune sera inhibit the binding of p70/p80 to DNA, and conversely, that binding of DNA to p70/p80 partially inhibits autoantibody binding in some cases (2). Thus, at least one of the regions predicted to have a possible role in DNA binding may also be an important autoepitope. Recent studies from our laboratory suggest that the majority of autoantibodies to p70 in most sera from patients with SLE are reactive with this region.[3,4]

The observation that antibodies eluted from the 70.34 fusion protein were specific for p70, and displayed no cross-reactivity with p80 suggests that the carboxyl-terminal 239 residues of p70 may not have extensive homology with p80, an interpretation that is also supported by the observation that p70 cDNA hybridized with a single poly(A)$^+$ RNA (Fig. 5). It seems unlikely, therefore, that p70 and p80 are derived from a single gene by an alternative splicing mechanism. The possibility that p70 is derived from proteolytic cleavage of p80 is also highly unlikely. The immunologic cross-reactivity of p70 and p80 previously reported (6) may therefore reflect a relatively short region of p80 amino acid sequence similarity, possibly near the amino terminus of p70. We have been unable to test this possibility due to difficulties obtaining fusion proteins containing the amino-terminal 115 amino acids of p70. Although clone 70.5 contains these residues and was obtained by antibody screening, only trace amounts of fusion protein were produced by *E. coli* Y1089 lysogenized by this clone. Furthermore, we have been unable to express this region in a variety of plasmid expression vectors.[4] The difficulty in expressing this region might relate to amino acid

[4] W. H. Reeves and Z. M. Sthoeger, unpublished observations.

sequences analogous to those that target certain proteins for rapid degradation in eukaryotic cells (48), or to low levels of synthesis and/or a high rate of degradation of the mRNA. Direct comparison of the sequence of p70 with that of p80, when available, may be necessary to localize the region(s) of immunologic similarity (6) between the two proteins. How autoimmunity to the p70 antigen develops, why it is closely linked to autoimmunity to p80, and whether the function of p70/p80 is related to the development of autoimmunity to the complex remain unanswered questions. The availability of the cloned autoantigens may be valuable in addressing these issues.

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